FINAL PERFORMANCE REPORT

Federal Aid Grant No. F15AF01149 (T-85-1)

Distribution, Abundance and Genetic Variation of the Prairie Speckled Chub

Oklahoma Department of Wildlife Conservation

January 1, 2016 – December 31, 2017

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State: Oklahoma **Grant Number:** F15AF01149 (T-85-1)

Grant Program: State Wildlife Grant

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Project Participants: Anuj Guruachary, PhD student.

Executive summary:
Macrhybopsis australis is a small cyprinid fish endemic to the upper Red River and its tributaries in the mixed-grass prairie region of Oklahoma and Texas which occurs in large creeks and small and large rivers where it prefers clean sand and gravel runs. *Macrhybopsis australis* appears to be extirpated from parts of its former range, including the Washita R. and the North Fork of the Red R. Although it was listed as common by Page and Burr (2011), local abundance may also be in decline in some localities. This study intends to conduct field surveys to assess the current distribution and abundance of *Macrhybopsis australis* in the upper Red River basin of Oklahoma, and to assess genetic variation in *M. australis* to evaluate the status of the species in terms of genetic and demographic viability. A total of 372 *Macrhybopsis australis* were collected at 18 out of 21 separate sites upstream from Lake Texoma. There was significantly lower abundance in the tributaries in areas of apparently suitable habitat, where prairie speckled chubs have historically been found. In contrast, within the mainstem of the Red River, all sites had significant numbers of speckled chubs. This likely reflects a larger area of suitable habitat maintained by higher discharge rates and with fewer barriers to dispersal. Our data indicate that there was over-representation of a few individuals in the genetic sequence reads. This usually stems from unequal DNA quantities among the samples before they were pooled for sequencing. This means that many sequences are from a few individuals and there were few sequences from many of the other individuals. As a result, not as many loci were found in all individuals as would typically be expected and read-depth (sequence reads per locus) was very low for many individuals. Results indicating that many localities had low abundance or absence of prairie speckled chubs raises concern about the long-term viability of populations in tributaries of the Red River. Maintenance of connectivity of small relatively isolated populations is necessary to prevent loss of genetic diversity as a small number of diverse populations can serve as source populations to provide allelic input into smaller populations.

I. BACKGROUND AND NEED:

The Prairie Speckled Chub, *Macrhybopsis australis*, is endemic to the upper Red River basin.

Macrhybopsis australis is a small cyprinid fish endemic to the upper Red River and its tributaries in the mixed-grass prairie region of Oklahoma and Texas (Eisenhour 2004, Miller and Robison 2004). It occurs in large creeks and small and large rivers where it prefers clean sand and gravel runs (Miller and Robison 2004). The species was until recently considered to be part of the variable and wide-ranging *Hybopsis aestivalis* complex. However, Eisenhour (1999, 2004), recognized five species in the Mississippi River and Gulf of Mexico drainages, three of which occur in Oklahoma. These include *Macrhybopsis australis* in the upper Red R., *M. tetranema* native to the upper Arkansas River basin, and *M. hyostoma* which occurs in both the Red and Arkansas drainages. Phylogenetic analysis of morphological data suggested that *M. australis* and *M. tetranema* are sister species and this group is closely related to *M. hyostoma* (Eisenhour 2004). *Macrhybopsis hyostoma* occurs mainly in lower reaches of the Red and Arkansas Rivers but it is sympatric with the other two species in small areas where the ranges overlap.

Altered stream flows may threaten *M. australis* populations.

Macrhybopsis australis appears to be well adapted to drought and highly variable plains stream flows, however disruption of natural flow regimes may have negative consequences for the species. Many of the streams in the range of *M. australis* may dry to isolated, salt-encrusted pools during summer. Under such conditions local populations may go extinct, followed by recolonization from surviving populations in adjacent parts of the range (Winston et al. 1991, Eisenhour 2004). However, dams and other anthropogenic barriers to dispersal may prevent recolonization of areas with locally extinct populations. In addition, dams, channelization, water removal and climate change may negatively affect the species if pools used as refuges during droughts were reduced, eliminated, or degraded (Matthews and Marsh-Matthews 2003). The species may be a flood-pulse spawner such that disruption of natural flow regimes may also inhibit reproduction by eliminating natural spawning cues (Eisenhour 2004).

The Prairie Speckled Chub, *Macrhybopsis australis*, appears to be in significant decline throughout much of its range.

Macrhybopsis australis appears to be extirpated from parts of its former range, including the Washita R. and the North Fork of the Red R. (Miller and Robison 2004, Winston et al. 1991). Although it was listed as common by Page and Burr (2011), local abundance may also be in decline in some localities. Current distribution and abundance data for *M. australis* are limited and population trends are unknown, making it difficult to identify management issues and establish effective corrective strategies. *Macrhybopsis australis* was recently petitioned for listing under the Endangered Species Act (WildEarth Guardians 2010), emphasizing the need for more comprehensive information to evaluate the status of the species within its geographic range in Oklahoma. A high priority is to assess the current distribution and abundance of the Prairie Speckled Chub in the upper Red River and its tributaries.

II. OBJECTIVES:

This project will conduct field surveys to assess the current distribution and abundance of Macrhybopsis australis in the upper Red River basin of Oklahoma. Genetic variation at nuclear and mitochondrial DNA markers within and among populations of M. australis and M. hyostoma will be assessed to evaluate the status of the species in terms of genetic and demographic viability. Comparison of allele frequencies in "pure" populations of each species and in contact zones between the two species will allow assessment of the extent of hybridization and species integrity.

III. METHODS:

Fish were collected by seine net from localities spanning much of the historical range of the speckled chub in the upper Red River and its tributaries. The number of individuals was noted from each collection and tissue samples for DNA extraction were taken. Some of the sampling involved fin clips, where fish were returned to the water unharmed. Some samples were provided as whole fish by Anthony Rodger from ODWC Streams Team collections.

DNA was isolated from each individual fish. We used an approach to assessing genetic variation and population structure known as ddRADseq (Peterson et al. 2012). Genomic "libraries" were prepared from each individual by incorporating adapters with "barcodes" and "indices" that allow DNA from each individual to be identified when samples are pooled to run on an Illumina sequencing instrument. We used the program Stacks (ref) to assemble reads from each individual (demultiplex) from the pooled the sequence data. *Structure* (Pritchard et al. 2000), which employs Bayesian statistical methods to estimate a series of population parameters, was used for within- and between-population analyses.

IV. RESULTS

Fish sample data.

Fish sample data are presented in Table 1. *Macrhybopsis australis* were collected at 18 out of 21 separate sites upstream from Lake Texoma. Three sites where no chubs were collected are included in Table 1 but were not given site numbers for later DNA analyses. Although speckled chubs were collected at most sites, numbers were quite low in the tributaries and west (upstream) of Hwy 283 (south of Altus, OK) in the mainstem of the Red River. This is near the transition to the Prairie Dog Town Fork where river discharge begins to be reduced. Two notable exceptions were our site 1 on the North Fork of the Red and site 4 on the Elm Fork of the Red where a larger number of speckled chubs were collected. Thus, there was significantly lower abundance in the tributaries in areas of apparently suitable habitat, where prairie speckled chubs have historically been found. In contrast, within the mainstem of the Red River, all sites had significant numbers of speckled chubs. This likely reflects a larger area of suitable habitat maintained by higher discharge rates and with fewer barriers to dispersal.

Genetic analyses.

Assembly of next-generation sequencing reads can be challenging where no reference genome sequence is available. The closest relative to *M. australis* with a high quality genome is

the zebrafish (*Danio rerio*) which has historically been considered to be in the same family (Cyprinidae). Typically, one would want a closer relative for reference assembly. We tried assembling both with the zebrafish reference genome and without a reference genome. It appears that the de-novo assembly was as effective as using the distantly related zebrafish reference genome.

Our data indicate that there was over-representation of a few individuals in the resulting set of sequence reads. This usually stems from unequal DNA quantities among the samples before they were pooled for sequencing. This means that many sequences are from a few individuals and there were few sequences from many of the other individuals. As a result, not as many loci were found in all individuals as would typically be expected and read-depth (sequence reads per locus) was very low for many individuals. For some individuals read depth was so low that an insufficient number of loci could be detected. Thus not all individuals that were collected yielded usable data (see Table 2). With a minimum read-depth of 3, an average of at least 900 independent loci per individual were recovered. This includes a total of 36,090 nucleotide sites examined with an average of 73 polymorphic sites.

Summary statistics of our genetic analyses are listed in Table 2. A primary function of the program *Structure* is to take the unstructured data and find some number of populations (*K*) that best explains the distribution of genetic variation. In this process, individuals are assigned to populations based on similarity of multi-locus or composite genotypes. With our data, the program found the optimal number of populations to be 7, and sample localities were grouped into populations that corresponded geographically to each tributary as well as the upper, middle and lower reaches of the Red River within the sampled area. This indicates there is relatively strong genetic differentiation among populations but relatively little variation within them. The number of polymorphic sites, which can be thought of as the number of variable loci or single nucleotide polymorphisms (SNPs), was largely proportional to the number of individuals analyzed per population. The observed level of heterozygosity (proportion of heterozygous loci) was larger than the level of heterozygosity expected based on the number of alleles present under Hardy-Weinberg equilibrium.

That many localities had low abundance or absence of prairie speckled chubs raises concern about the long-term viability of populations in tributaries of the Red River. Reconciliation of data indicating low abundance with relatively high genetic diversity suggests a scenario where populations in the North Fork, Salt Fork and Prairie Dog Town Fork have undergone recent reductions in population size at many localities. Populations with recent reductions in population size may continue to harbor a larger number of alleles and hold genetic diversity indicative of much larger populations. This is because there is a lag between reduction in census size and a reduction of genetic diversity by genetic drift. Thus small populations may harbor substantial genetic variation that reflects their former population size.

Maintenance of connectivity of small relatively isolated populations is necessary to prevent loss of genetic diversity as a small number of diverse populations can serve as source populations to provide allelic input into smaller populations. Recolonization of populations that have undergone local reduction or extinction from larger source populations represents a metapopulation that is characterized by gradual genetic divergence with occasional gene flow to elevate genetic diversity. Local tributary populations may not be able to persist where there are barriers to dispersal and gene flow. Alternatively, the larger populations in the Red River mainstem do not appear to be threatened by a lack of connectivity.

Hybridization of *M. australis* with *M. hyostoma* may contribute to divergence of *M. australis* populations in the lower reaches of the Red River near Lake Texoma relative to upstream populations due to introgression of *M. hyostoma* alleles. This could ultimately result in breakdown of the species integrity of *M. australis* if genomic introgression becomes widespread. We cannot comment on the current potential for this result, as very few *M. hyostoma* could be positively identified in our samples. As a result we could not identify putative *M. hyostoma* alleles and the level of hybridization remains unknown.

V. RECOMMENDATIONS

1. Continued monitoring is needed to assess stasis or changes in abundance and genetic structure of these prairie speckled chub populations, particularly in the North Fork, Salt Fork and Prairie Dog Town Fork of the Red River.
2. We recommend that to the extent possible, water levels and flow regimes be maintained without additional barriers to dispersal of these fish.

VI. SIGNIFICANT DEVIATIONS

There have been no significant deviations.

VII. EQUIPMENT

No equipment exceeding $5,000 was purchased for this project.

VIII. PREPARED BY: Richard Broughton, University of Oklahoma

DATE: May 9, 2018

APPROVED BY:

Fisheries Division Administration
Oklahoma Department of Wildlife Conservation

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Table 1. Site and abundance data for prairie speckled chub collections in the upper Red River drainage. Sites are listed in ascending order from tributaries to the main stem, and from upstream to downstream

Site number	GPS coordinates	Stream	Locality	# fish	# times visited/collected
1	34.865812, -99.311120	North Fork Red	Hwy 44 bridge	18	1
2	34.637870, -99.103725	North Fork Red	~12 mi E Altus, Hwy 62 bridge	1	2
3	34.507190, -99.208513	North Fork Red	Hwy 5 bridge	5	1
4	34.926419, -99.502334	Elm Fork Red	Hwy 283 bridge	59	3
5	34.859225, -99.335799	Elm Fork Red	private land, near N Fk	1	1
	34.858549, -99.509425	Salt Fork Red	~1 mi S Mangum, Hwy 34 bridge	0	1
	34.637765, -99.408644	Salt Fork Red	~4 mi W Altus, Hwy 62 bridge	0	1
6	34.478976, -99.383030	Salt Fork Red	Elmer Rd ~1.5 mi W Elmer	8	1
7	34.577741, -99.957533	Prairie Dog Town Fork	Hollis Rd bridge	5	1
8	34.414064, -99.735599	Prairie Dog Town Fork	Hwy 6 bridge	2	2
9	34.457593, -99.379790	Red River	~3 mi SW Elmer, no bridge	14	1
10	34.209653, -99.082112	Red River	Hwy 183 bridge	48	3
	34.391154, -98.722806	Cache Creek	~5.5 mi SW Chattanooga, Hwy 5 bridge	0	1
11	34.109304, -98.533277	Red River	I-44 bridge	46	2
12	33.878632, -97.934273	Red River	Hwy 81 bridge	24	1
13	33.953923, -97.711485	Red River	~20 mi SE Waurika, no bridge	30	2
14	33.917089, -97.485578	Red River	~2 mi SE Courtney, no bridge	22	1
15	33.823091, -97.437313	Red River	~4 mi S Leon, no bridge	21	1
16	33.866441, -97.260474	Red River	~10 mi SW Marietta, no bridge	20	1
17	33.912017, -97.198963	Red River	~7 mi SW Marietta, no bridge	22	1
18	33.727802, -97.159594	Red River	I-35 bridge	26	1

Table 2. Genetic parameters for prairie speckled chubs from sampled localities. Colors distinguish genetically distinct populations indicated by *Structure* analysis.

Site number[a]	Stream	Assigned pop[b]	# indiv w/ data[c]	# polym. Sites[d]	Obs. Het.[e]	Exp. Het.[e]
1	North Fork Red	1	14	12	0.1643	0.0839
2	North Fork Red	1	0			
3	North Fork Red	1	2			
4	Elm Fork Red	2	30	43	0.1715	0.0887
5	Elm Fork Red	2	1			
6	Salt Fork Red	3	8	71	0.2616	0.1445
7	Prairie Dog Town Fork	4	5	40	0.1653	0.0832
8	Prairie Dog Town Fork	4	2			
9	Red River	5	12	112	0.191	0.1414
10	Red River	5	36			
11	Red River	5	36			
12	Red River	6	11	147	0.2225	0.1605
13	Red River	6	17			
14	Red River	6	14			
15	Red River	6	21			
16	Red River	7	18	85	0.1858	0.1269
17	Red River	7	16			
18	Red River	7	21			

[a] Sites from Table 1.
[b] Populations as detected by the program *Structure*.
[c] Listed are the actual number of individuals for which genetic data were obtained.
[d] This column lists the number of polymorphic sites for each assigned population among all loci (also the number of variable loci or SNPs).
[e] The two right-most columns list observed and expected heterozygosity respectively for each assigned population.